Meta-analysis of two RNAseq datasets to determine diagnostic biomarkers and drug target candidates for periodontitis

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Abstract

Periodontitis is a chronic inflammatory oral disease that affects approximately 47% of adults aged 30 or older in the United States. It is a more severe form of gingivitis that is characterized by alveolar bone loss and destruction of periodontal ligaments. Antibiotic treatments only treat a portion of the problem as the host immune system creates an inflammatory environment in which soft tissue and alveolar bone destruction occurs. In this study, we sought to identify diagnostic biomarkers and the mechanistic drivers of inflammation in periodontitis in order to identify drugs that may be repurposed to treat chronic inflammation. We performed a meta-analysis with two RNAseq datasets retrieved from NCBI Gene Omnibus. RNAseq analysis, SPIA, protein-protein interaction analysis, and drug target analysis were performed to determine the mechanistic drivers of inflammation in periodontitis and potential drug targets. We identified 78 differentially expressed genes, 10 significantly impacted signaling pathways, and 10 hub genes in periodontal gingival tissue. We identified the top 10 drugs that may be repurposed into treating periodontitis. The efficacy of these drugs in treating periodontitis has yet to be investigated. However, our analysis indicates that these drugs may serve as potential therapeutics to treat inflammation in periodontitis gingival tissue.

Introduction

Periodontitis is a chronic inflammatory oral disease that affects approximately 47% of adults aged 30 or older in the United States (Eke 2009). In 2018, the direct and indirect economic burdens of periodontal disease within the U.S. was estimated to be about $154.06 billion and about €158.64B in Europe (32 European countries) (Botelho 2021). It is a more severe form of gingivitis that is characterized by the recession of gums, alveolar bone loss, destruction of periodontal ligaments, and tooth decay in addition to swollen and bleeding gums (CDC). Periodontitis is caused by microbial dysbiosis within the periodontal pockets surrounding teeth, typically a result of improper oral hygiene. Interactions between pathogenic bacteria (primarily gram-negative Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola (Holt 2000)) and the host immune system creates an inflammatory environment which leads to the observed pathology. If left untreated, the continued destruction of periodontal ligaments and alveolar bone may result in reduced tooth support and eventually tooth loss (Yucel 2013). Although prevention of this disease is possible through proper oral hygiene practices, such as brushing and flossing teeth daily, periodontal disease is still considered to be an important public health problem in the United States (U.S. Department of Health, Eke 2014).

Medical interventions are required during severe periodontitis, including deep cleaning of tooth root surfaces to remove bacterial biofilms, antibiotics to kill pathogenic bacteria that are present, and corrective surgeries (CDC). Medications directed at treating bacterial dysbiosis, such as antibiotics, only addresses a portion of the problem in periodontitis as the host immune response has a critical role in the production of inflammatory mediators that results in the observed soft tissue damage (Yucel 2013).

The underlying mechanistic drivers of inflammation in gingival epithelial cells and/or resident and recruited immune cells may serve as potential drug targets to mitigate the inflammatory response and consequently reduce tissue destruction in periodontitis. In this study, we sought to identify relevant inflammatory markers and potential drug targets that could be modulated to reduce the inflammatory response in human periodontitis. To identify these potential inflammatory markers and targets, we used RNA analysis, signaling pathway enrichment analysis (SPIA), protein-protein interactions network, and drug target analysis. Herein, we report the identification of 10 potential drug targets which may serve as potential therapeutics to reduce inflammation and tissue destruction in periodontitis.

Methods

RNAseq Analysis

26 periodontal disease SRA files which represent 22 periodontitis RNA samples and 22 healthy RNA samples were downloaded from two series (GSE173082 and GSE80715) retrieved from NCBI Gene Expression Omnibus (GEO) (Table 1).

RNA samples from the GSE80715 series were isolated from 9 periodontal healthy patients and 4 periodontitis patients. Patient information about the RNA samples from the GSE173082 series was not provided by the investigators on NCBI GEO. Periodontitis and healthy RNA samples were analyzed using the ARMOR Snakemake-based workflow within a dedicated Conda environment, as described by Orjuela (Orjuela 2019). Briefly, quality control was performed on all RNA reads using fastQC. Reads with sufficient quality scores were trimmed using TrimGalore prior to mapping and quantification to the human GRCh38 reference transcriptome using Salmon (Patro 2017). Transcript quantifications from Salmon were then summarized at the gene level quantifications prior to performing differential gene analysis using EdgeR (Robinson 2010).

Signaling pathway analysis

All Ensembl Gene IDs from edgeR were converted into their corresponding NCBI Gene IDs utilizing the BioMart database. These NCBI Gene IDs were then combined with the fold-change and p-values from edgeR to generate the input for the signaling pathway impact analysis (SPIA) algorithm (Tarca 2009).

Drug Target Analysis

The SPIA output file was used as the input for drug target analysis which was performed using the Pathways2Targets R script that had previously been developed (Scott 2021). Briefly, all gene IDs from each of the pathways identified in the SPIA out file were retrieved and converted into protein IDs. Those protein IDs were then used to search in the public OpenTargets.org database for known drug targets (Ochoa 2021).

Protein-protein interactions analysis

Drug targets were used to construct the PPI network using the Search Tool for the Retrieval of Interacting Genes (STRING) (Szklarczyk 2021) database (Version 11.5, http://string-db.org/). The PPI network was visualized in the Cytoscape (Otasek) software (Version 3.9.0), and cytoHubba and MCODE plugins were used to calculate the degrees of nodes and to identify significant modules. The top ten transcripts with the highest degrees were identified as hub genes.

Results

Out of a total of 15,699 genes that were detected, we identified 78 differentially expressed genes (DEG) from our RNAseq analysis. 52 of the DEGs were identified as immunoglobulin domains with four additional pseudogenes detected. The remaining 22 genes contributed to various functions, including gene transcription, metabolite transport, toll-like receptor signaling, chemokine secretion, and endoplasmic reticulum stress (Table 2).

We next used the SPIA algorithm to determine whether any known intracellular signaling pathways were enriched in DEGs. SPIA identified ten pathways that were significantly impacted in periodontitis gingival tissues listed in Table 3.

We visualized the protein-protein interaction (PPI) network of drug targets that mapped back to all of the statistically significant signaling pathways identified as playing a role in periodontitis gingival tissue. Our initial PPI network, which we constructed using the online STRING database, consisted of 7,462 edges and 304 nodes. Using cytoHubba, we reduced the initial network to the top ten "central hub" genes based on degrees, or number of neighbors. Those central hub genes are listed in Figure 1.

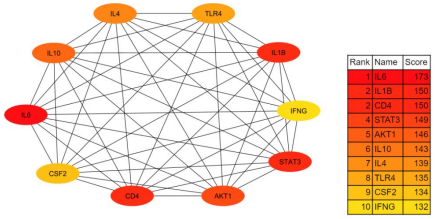
We then wanted to determine whether we could apply the signaling pathway results to predict existing drugs that could be repurposed as potential therapeutics. Using the output from SPIA, we were able to identify 335 protein targets with known drugs that target these proteins. Out of those 335 targets, we focused on the top 10 drugs that targeted our hub genes since they would be most likely to reverse the observed periodontitis phenotype (Table 4). We did, however, include a drug that targeted interleukin 17 receptor A (IL17RA). It wasn't one of the hub genes, but it was one of the drug targets identified in our analysis. IL17A has been associated with bone resorption in periodontitis and is produced by CD4+ type 17 T helper cells.

Table 1. Summary of two individual datasets of periodontitis

GEO gene set ID	GSE173082	GSE80715
Title	Differential DNA methylation & mRNA expression in gingival tissues in periodontal health and disease	Transcriptome analysis of chronic periodontitis patients' gingival tissue
Platform	Illumina HiSeq 4000	Illumina HiSeq 2000
Library Construction Protocol	Gingival tissue samples were harvested in conjunction with an invasive oral surgical procedure required for the participant's oral care after administration of local anesthesia. Poly-A pull-down was carried out to enrich mRNAs from total RNA samples (200ng-1g per sample) followed by library preparation using the Illumina TruSeq RNA prep kit.	Frozen tissues were disrupted in the lysis solution of miVana RNA isolation kit (Thermo Fisher Scientific) using disposable pestle grinder system (Thermo Fisher Scientific). After purification of mRNA molecules by poly-T oligo-attached magnetic beads followed by fragmentation, the RNA of approximately 300 bp size was isolated using gel electrophoresis. The cDNA synthesis and library construction was performed using the Illumina TruSeq RNA sample preparation kit (Illumina, San Diego, CA) following the manufacturer's protocol.
Sample Type	Single End	Paired End
Diagnostic criteria	Not recorded	On the basis of clinical and radiographic criteria, periodontitis-affected site had a probing depth of ≥ 4 mm, clinical attachment level of ≥ 4 mm, and bleeding on probing
Sample Prep	Not recorded	The size of 3 mm2 gingival biopsies were obtained from the marginal gingiva during periodontal flap surgery and immediately stored in RNAlater solution (Thermo Fisher Scientific, Waltham, MA) at −70oC after removal of blood by brief washing in phosphate buffered saline.
Number of healthy samples vs. periodontitis samples	12 vs 12	10 vs 10
Healthy Tissues	Not recorded	9 periodontal healthy patients with pocket depth <4 mm
Periodontitis	Not recorded	4 periodontitis patients with pocket depth of 4-6 mm; 3 severe periodontitis patients with pocket depth of 7 mm or deeper
PubMed ID	Not published	27531006

Table 3. Significantly impacted signaling pathways

	Name	pSize	NDE	pNDE	tA	pPERT	pG	pGFdr	pGFWER	Status
1	Cytokine-cytokine receptor interaction	177	39	1.27E-05	13.49	1.20E-03	2.90E-07	5.01E-05	5.01E-05	Activated
2	Staphylococcus aureus infection	29	13	3.66E-06	9.22	1.55E-01	8.72E-06	7.55E-04	1.51E-03	Activated
3	Natural killer cell mediated cytotoxicity	95	23	1.70E-04	47.51	1.28E-02	3.06E-05	1.47E-03	5.29E-03	Activated
4	Chemokine signaling pathway	157	30	1.52E-03	31.64	1.60E-03	3.39E-05	1.47E-03	5.87E-03	Activated
5	Osteoclast differentiation	108	25	1.94E-04	13.41	6.88E-02	1.63E-04	5.65E-03	2.83E-02	Activated
6	Leukocyte transendothelial migration	76	19	3.94E-04	19.35	4.92E-02	2.30E-04	6.63E-03	3.98E-02	Activated
7	Keratinization	90	27	6.00E-07	-1.90	2.00E-01	2.03E-06	1.43E-03	1.43E-03	Inhibited
8	Innate Immune System	533	101	4.65E-05	67.93	7.80E-02	4.90E-05	1.01E-02	3.47E-02	Activated
9	Assembly of collagen fibrils and other multimeric structures	45	12	2.51E-03	7.31	1.60E-03	5.38E-05	1.01E-02	3.80E-02	Activated
10	Formation of the cornified envelope	62	19	1.96E-05	-1.89	2.16E-01	5.71E-05	1.01E-02	4.03E-02	Inhibited



Rank	Name	Score
1	IL6	173
2	IL1B	150
2	CD4	150
4	STAT3	149
5	AKT1	146
6	IL10	143
7	IL4	139
8	TLR4	135
9	CSF2	134
10	IFNG	132

Figure 1. Protein-protein interaction network of top 10 hub genes and their rankings by degrees method.

Table 2. Differentially Expressed Genes identified in periodontitis gingival tissue

	Ensembl Gene ID	Symbol	Description	logFC	logCPM	PValue	FDR
1	ENSG00000099958	DERL3	Derlin 3	3.92	4.40	3.45E-05	2.92E-02
2	ENSG00000170476	MZB1	Marginal zone B and B1 cell specific protein	3.98	5.29	4.48E-05	2.92E-02
3	ENSG00000153208	MERTK	MER proto-oncogene, tyrosine kinase	1.58	1.32	4.56E-05	2.92E-02
4	ENSG00000183508	TENT5C	Terminal nucleotidyltransferase 5C	3.11	5.28	6.44E-05	2.98E-02
5	ENSG00000198794	SCAMP5	Secretory carrier membrane protein 5	2.67	2.54	6.79E-05	3.03E-02
6	ENSG00000137265	IRF4	Interferon regulatory factor 4	3.14	4.02	7.33E-05	3.03E-02
7	ENSG00000061656	SPAG4	Sperm associated antigen 4	3.28	1.63	7.87E-05	3.03E-02
8	ENSG00000112936	C7	Complement C7	2.46	-0.06	8.31E-05	3.03E-02
9	ENSG00000100219	XBP1	X-box binding protein 1	1.81	7.87	8.99E-05	3.05E-02
10	ENSG00000065413	ANKRD44	Ankyrin repeat domain 44	1.41	3.31	9.72E-05	3.05E-02
11	ENSG00000117322	CR2	Complement C3d receptor 2	5.14	0.83	1.19E-04	3.39E-02
12	ENSG00000189233	NUGGC	Nuclear GTPase, germinal center associated	2.02	0.83	1.25E-04	3.48E-02
13	ENSG00000134265	FKBP11	FKBP prolyl isomerase 11	2.02	4.53	1.34E-04	3.57E-02
14	ENSG00000102096	PIM2	Pim-2 proto-oncogene, serine/threonine kinase	2.60	4.33	1.58E-04	3.94E-02
15	ENSG00000198035	ENTPD7	ectonucleoside triphosphate diphosphohydrolase 7	0.70	4.47	1.70E-04	4.15E-02
16	ENSG00000130768	SMPDL3B	Sphingomyelin phosphodiesterase acid like 3B	1.99	1.39	1.76E-04	4.20E-02
17	ENSG00000101194	SLC17A9	Solute carrier family 17 member 9	2.46	1.75	1.84E-04	4.25E-02
18	ENSG00000153162	BMP6	Bone morphogenetic protein 6	1.89	2.26	1.95E-04	4.30E-02
19	ENSG00000073849	ST6GAL1	ST6 beta-galactoside alpha-2,6-sialyltransferase 1	2.12	5.13	1.97E-04	4.30E-02
20	ENSG00000198854	C1orf56	chromosome 1 open reading frame 56	-3.78	1.50	2.03E-04	4.36E-02
21	ENSG00000122188	LAX1	Lymphocyte transmembrane adaptor 1	2.77	2.44	2.33E-04	4.81E-02
22	ENSG00000091490	SEL1L3	SEL1L family member 3	2.15	4.70	2.45E-04	4.92E-02

Table 4. List of drug targets.

	Target Symbol	Target Name(s)	Drug ID	Drug Name	Is FDA Approved	Highest Clinical Trial Phase
1	IL6R; IL6ST	Interleukin 6 receptor; Interleukin 6 cytokine family signal transducer	CHEMBL3833307	Satralizumab	TRUE	4
2	TNFSF11	TNF superfamily member 11 (RANKL)	CHEMBL1237023	Denosumab	TRUE	4
3	IFNAR2	Interferon alpha and beta receptor subunit 2	CHEMBL1201563	Interferon Beta-1B	TRUE	4
4	IL17RA	Interleukin 17 receptor A	CHEMBL1742996	Brodalumab	TRUE	4
5	TLR4	Toll like receptor 4	CHEMBL225157	Resatorvid	FALSE	3
6	IL6	Interleukin 6	CHEMBL2108569	Clazakizumab	FALSE	3
7	IL1B	Interleukin 1 beta	CHEMBL1743026	Gevokizumab	FALSE	3
8	TGFBR1	Transforming growth factor beta receptor 1	CHEMBL2364611	Galunisertib	FALSE	2
9	CSF2RB	Colony stimulating factor 2 receptor subunit beta	CHEMBL1743039	Mavrilimumab	FALSE	2
10	CSF2	Colony stimulating factor 2	CHEMBL2109430	Gimsilumab	FALSE	2

Conclusions

The efficacy of these drugs in treating periodontitis has yet to be investigated. However, our analysis indicates that these drugs may serve as potential therapeutics, either individually or in combination, to treat inflammation in periodontitis gingival tissue. All of these drugs in some way mitigate the inflammatory response or bone resorption via their mechanism of action. As noted above, reducing inflammation induced by periodontitis may also reduce the severity of other systemic diseases by reducing the amount of inflammatory cytokines and activated immune cells that circulate or migrate to other tissues throughout the body. Thus, it is worth investigating the efficacy of these drugs in reducing the inflammation in the oral cavity during periodontitis in periodontitis animal models.

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